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Evolutionary relatedness of human pathogenic bacteria based on conserved and structural gene sequences

Manish Kumar and Raghvendra Saxena
Amity Institute of Biotechnology, Amity University Madhya Pradesh, Maharajpur, Dang, Gwalior India 474005
*corresponding author (mkumar@gwa.amity.edu)

Abstract

Phylogenetic tree is a visual representation of relations between organisms, species and gene sequences. It is a study to represent evolutionary history of living organism using tree like figure. Our indigenous environment contains pathogens which severely affect the host species. Most preferable host is a human body, as it provides nutrients, warm and moist environment which helps pathogens to survive. A molecular characterization was used to point out the pathogenic bacteria at the molecular level without any disturbance of environmental or physical conditions. Molecular characterization started with retrieval of nucleotide sequences of eleven pathogenic bacteria on the basis of 16S rRNA and DNA gyrase gene from NCBI in FASTA format and sequence were aligned by CLUSTALW and tree was constructed by MEGA software. Phylogenetic analysis of eleven pathogenic bacteria on the basis of 16S rRNA and DNA gyrase gene has contributed to illustrate the closely and distantly relatedness of disease causing bacteria and comparison of the both phylogenies interpreted that positions occupied by pathogenic bacteria are different in both the evolutionary trees. As stated by many researchers phylogenetic methods are reliable technique for identification.

KEYWORDS – Phylogenetic tree, Pathogenic bacteria, Molecular characterization, MEGA, 16S rRNA, DNA gyrase.

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Introduction

As in the period of Charles Darwin, it is said that about remembering the structure of phylogeny whether it is for individual microorganism or for group of microorganism with their respective species is important (the 16S rRNA sequences convert to branching sequences to represent there evolutionary history). Unluckily, it's very complex form of sequence analysis. Charles Darwin has given two postulates for biological evolution or growth. (1) Innovative evolution occurs by the time and- (2) Dissimilarity in evolution represented by individual according to time. The term 'phylogeny' is defined as a history of an organism which shows evolution. The structure represents evolutionary relationship among various organisms called as phylogenetic tree (Morrison 2010). Investigation and test of evolution can be identifying by phylogenetic tree. Phylogenetic tree represents two types of trees *viz.* rooted or un-rooted tree. The phylogeny describes group evolution or group changes in organism. The phylogenetic tree represents phenotypic and genotypic characters (Stephen et al. 2013). Comparative phylogeny method or phylogenetic comparative method (PCMs) permit us to understand about the organismal evolutionary relationship with their common ancestor and another approach is given that it shows diversification or variation, it represents both evolutionary relationship and diversification at same time. Phylogenetics is the surrounding to understand the biodiversity. Phylogenetic comparative method contains a company of numerical data methods for representation of the information which is the combination of two statistics: First relation of species on the basis of their gene and second based on the peer or current characters. Some PCMs help to find history with some geological record or fossils. PCMs are the method of separate set in phylogenetics (Cornwell and Nakagawa 2017). Construction of phylogenetic tree can be enabling by various software. Tree can be constructed by 'neighbour joining method' who represents closely or distantly relationship with peer organism and other method is 'maximum likelihood method' which represents the likelihood or similarity and dissimilarity in characters and functions with the peer organism (Talib et al 2016). In case of pathogen it is beneficial and interesting to know about the disease evolution at genotypic and phenotypic level. It justify about the past and present of disease. The word 'pathogen' is originated from Greek word '*patho*' means ''disease'' and *'genes'* means ''born of 'derived in 1880. Pathogens can be virus and bacteria which cause infection or disease to their host, basically pathogens are an infectious causing agent. Pathogen can be classified on the basis of morphological resemblance (site of

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infection, way, their host) or resemblance of disease (symptoms, cure process) (Morrison 2010). Pathogenicity is the job of microorganism to cause infection in host's body. The human body is a compound and develop structure. Human body consist of total 1013 cells and approx 1014 normal flora or microorganism which live on surface of host (Alberts et al. 2002). Pathogenic bacteria can enter into human body by different source of transmission. The bacterium reflects it individual pathogenicity. Bacteria are classified into three classes on the basis of their disease causing ability – Primary pathogens are those cause disease to every individual (e.g., *Shigella spp.* isolated from human and animal faeces). Opportunistic pathogens are cause disease to host with immunodeficiency (e.g., *Escherichia coli* isolated from urine from the host who is suffering from urinary tract infection). Attenuated bacteria are those are converted to vaccines to resist the disease (e.g., Tuberculosis –*BCG* live attenuated bacteria *Mycobacterium bovis)* (Peterson 1996). Pathogenic bacteria which cause foodborne illness, pneumonia, gastroenteritis, diarrhoea are *Salmonella enterica, Shigella sonnei, Vibrio cholerae, E.coli, Legionella pneumophila, Yersinia enterocolitica, Staphylococcus hominis, Streptococcus pneumoniae, mycobacterium tuberculosis, campylobacter jejuni* and *Chlamydia pneumonia*. The identification of pathogenic bacteria is important in many aspects, benefits to doctors to treat their patients accurately and on the basis of research aspects we are able to know about their morphology, characters, phylogeny, structure, evolutionary changes, relationships, disease, symptoms, diagnosis, benefits or loss (Pandey et al.2019). First approach to pathogenic bacteria identification – Close observation is important when the infection is communicated, comprehensive. In USA (Active Bacterial Core Surveillance) is set up in 1995 and in EU (European Centre for Disease Prevention and Control) in 1996, WHO also reported the surveillance in 2014 across the world (World Health Organization (WHO). Second approach is to identify or detect about its diagnosis and to check that "is it bacterial infection or any other infection." Third approach is to check that bacterial strain is susceptible or resistant. Fourth approach is classification and identification method. Classification - Making a list of organism on the basis of characteristics and placing them with their related species. Identification – To identify the unknown bacteria on the basis of character sticks. Identification techniques take place in two aspects: 1. Biochemical characterization 2. Molecular characterization. The biochemical characterization gives morphological information. In clinical microbiology labs the traditional techniques are used to detect most of the sample of pathogenic bacteria strain. Firstly the isolation of bacteria is

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done then we move to further process of biochemical tests. The various biochemical tests are performed: IMViC test is considered a group test for an individual bacterium. A coli form group test which identify in such a way e.g. aerobic or anaerobic, facultative, gram positive or negative, rod or cylindrical shape. *Yersinia enterocolitica, Staphylococcus aureus, Klebsiella pneumonia* has been detected by this test; agar-based media is the media which support the growth of pathogenic bacteria. The specific culture is important to identify pathogens, (e.g. blood agar), MALDI- TOF MS (matrix assisted laser desorption/ionization – time of flight mass spectrometry) has been done to identify *Bacillus anthracis,* fatty acid profiles test is used because in bacterial cell wall fatty acids are essential components to determine the lipid structure A and lipid structure B, flow cytometry is process which used to define the chemical and physical structure of the cell or particles. The sample containing cell is run in flow cytometry instrument (Picot et al. 2012) Molecular characterization method is based on genome and provides genotypic information. Molecular characterization is measured by DNA sequencing. It is the identification at the molecular level without any interruption of environment, physiological state, development or growth. It represents the serology approach, two check relationship between two unknown bacteria. The molecular methods are: Phylogenetic analysis – Analysis based on 16S rRNA of bacteria. Phylogenetic analysis is done by some software e.g. MEGA –X. Phylogenetic tree construction takes place, nucleic Acid and hybridization techniques – In this detection nucleic acids DNA or RNA which are single stranded are permitted to form hybrid, by interacting with complexes, PCR – (Polymerase chain reaction) it is a universal method, it permit accurate identification of bacteria. First we have to obtain 16S gene sequenced, against the bacterial DNA databases then bacteria is identified, DNA fingerprinting – The process of analysis of DNA to identify individual or species. DNA profiling is basically a forensic experiment. (Elijah. et.al 2014), DNA microarrays technique- It is also known as the DNA chip technology. It is a laboratory tool used to detect the expression of thousand genes at the same time (Taub et.al 1983), whole genome sequencing – It is process to describe whole DNA. It is mostly used in medical researches and clinical practices (Gilissen 2014).

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Materials and methods

Retrieval of nucleotide sequence of 16S rRNA gene of pathogenic bacteria from NCBI

NCBI is the national centre for Biotechnology information; it is a collective series of databases applicable to biotechnology and biomedicine. Bioinformatics tools are also available in this source. It contains various databases for Genbank for nucleotide sequences, Pubmed for research papers (Stephen 2007). To compare closely or distantly relationship among organism, to check diversification, evolution among organism, the nucleotide sequence need to be downloaded. The procedure to download nucleotide sequence is as follow:

- Go to home page of NCBI.
- Then nucleotide as a resource was selected by clicking on 'all database.'
- Then typed the name of pathogenic strain along with the gene 16S rRNA.
- There after various results got visible on the site.
- After clicking on FASTA, 16S rRNA sequence became visible.
- Then sequences were copied successfully along with accession number and saved in a notepad file (Adhikari et al. 2015).

Retrieval of nucleotide sequence of DNA gyrase gene of pathogenic bacteria from NCBI

DNA gyrase can be known as simply gyrase also. DNA gyrase is a tetramer enzyme contain 2gyr A ('A') and 2gyr B ('B') subunit. Gyr B is a housekeeping gene and plays a chief role in replication. DNA gyrase is an enzyme of topoisomerase class and subclass of topoisomerase type 2. The phylogenetic relationship cannot justify on the basis of 16S rRNA only, that's why comparison is essential. The DNA gyrase gene sequence was also retrieved from NCBI. The procedure to download nucleotide sequence is as follow:

- Go to home page of NCBI.
- Then nucleotide as a resource was selected by clicking on 'all database.'
- Then typed the name of pathogenic strain along with the gene gyrase.
- There after various results got visible on the site.
- After clicking on FASTA, gyrase sequence became visible.
- Then sequences were copied successfully along with accession number and saved in a notepad file (Adhikari et al. 2015).

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Alignment of sequences by CLUSTALW

Widely used system is a CLUSTAL W for aligning any number of sequences. The method of aligning multiple sequences CLUSTALW has progressive alignment tool. In this tool closely related sequences or similar sequences are those, that contain best score are aligned first. Then progressively the distant or dissimilar sequences are aligned only when global alignment is obtained. CLUSTAL W performs very well and clean task. The algorithms start calculating a distant matrix between the pair of sequence based on pair wise sequence alignment. The scores are calculated using pair wise alignment framework for DNA and protein sequences The method for alignment is: installed a MEGA software in window then open the mega software, click on alignment, select the alignment explorer and create new alignment, then click on insert sequence from file and upload your sequences, click on ClustalW tool a multiple sequence alignment tool associate with the software, by considering all defaults parameters click on OK, click on save session by giving name on file (Higgins et al. 1994).

Construction of phylogenetic tree by MEGA 10 (MEGA X) Software

MEGA software is also known as molecular evolutionary genetic analysis developed by Penn State University. It is used for conducting statistical analysis for molecular evolution and for constructing a phylogenetic tree. It includes many sophisticated tool in which CLUSTAL W is one of them. CLUSTAL W align the sequences in two methods, first is multiple sequence alignment in which more than two sequences are aligned and second one is pair wise sequence alignment in each pair of sequence is aligned on the basis of parameters DNA and protein sequences. After the alignment of sequences; construction of phylogenetic tree takes place. Phylogenetic tree can be constructed by neighbour joining method or distanced based method is created by Sitou and Nei in (1987). This method explains whether microorganism is closely related or distantly related or sharing of the common ancestors. The method for constructing phylogenetic tree is maximum likelihood method or character based method which explains the likelihood in characters, functions with peer microorganisms. Construction of Phylogenetic tree on the basis of 16S rRNA and DNA gyrase is done in steps: first alignment is done in MEGA 10 software with help of align tool insert a sequences saved in file, after the alignment save the file in MEGA format, then click on phylogeny and selected the bootstrap neighbour joining method for construction of phylogenetic tree, the tree appears in another window can be saved in pdf, can be copied and can be saved in MEGA format also (Chenna et al. 2003).

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Result and Discussion

The nucleotide sequence alignment of 16S rRNA sequences

The 16S rRNA gene nucleotide sequences of *E.coli, Campylobacter jejuni, Salmonella enterica, Yersinia enterocolitica, Chlamydia trachomatis, Mycobacterium tuberculosis, Shigella sonnei, Staphylococcus hominis, Streptococcus pneumoniae, Vibrio cholerae, Legionella pneumophila* were retrieved from NCBI in FASTA format. The nucleotide sequences along with their accession number were downloaded and saved in notepad file. Alignment of 16S rRNA sequence has been done by CLUSTALW present in MEGA software by default.

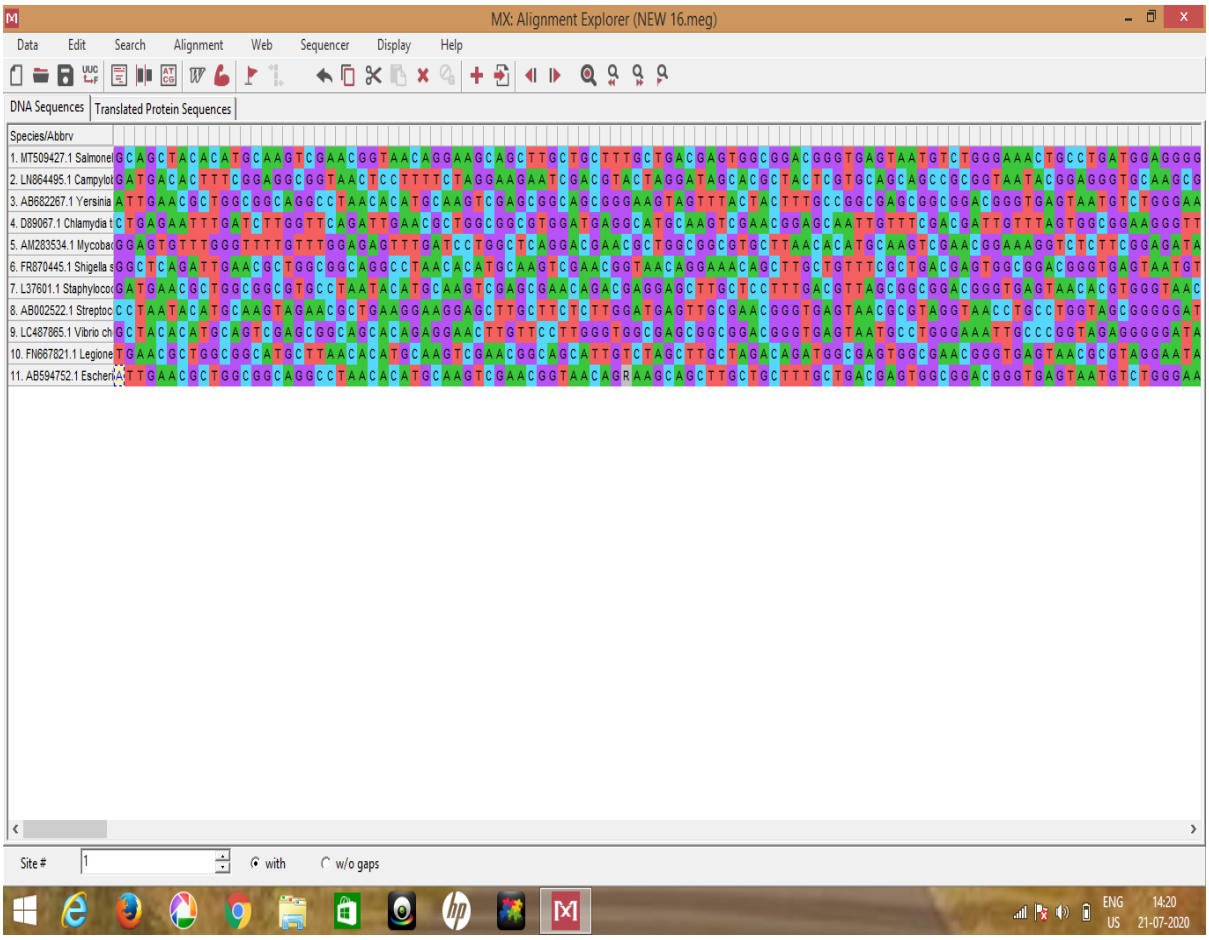

Fig 4.1- The sequence alignment of 16S rRNA gene sequences of pathogenic bacteria by CLUSTALW.

The nucleotide sequence alignment of DNA gyrase gene sequences

Apart from 16S rRNA gene nucleotide sequences DNA gyrase gene nucleotide sequences of *E.coli, Campylobacter jejuni, Salmonella enterica, Yersinia enterocolitica, Chlamydia trachomatis, Mycobacterium tuberculosis, Shigella sonnei, Staphylococcus hominis, Streptococcus pneumoniae, Vibrio cholerae, Legionella pneumophila* were retrieved from NCBI in FASTA format. The nucleotide sequences along with their accession number were downloaded and saved in notepad file. Alignment of DNA gyrase sequences has been done by CLUSTALW present in MEGA software by default.

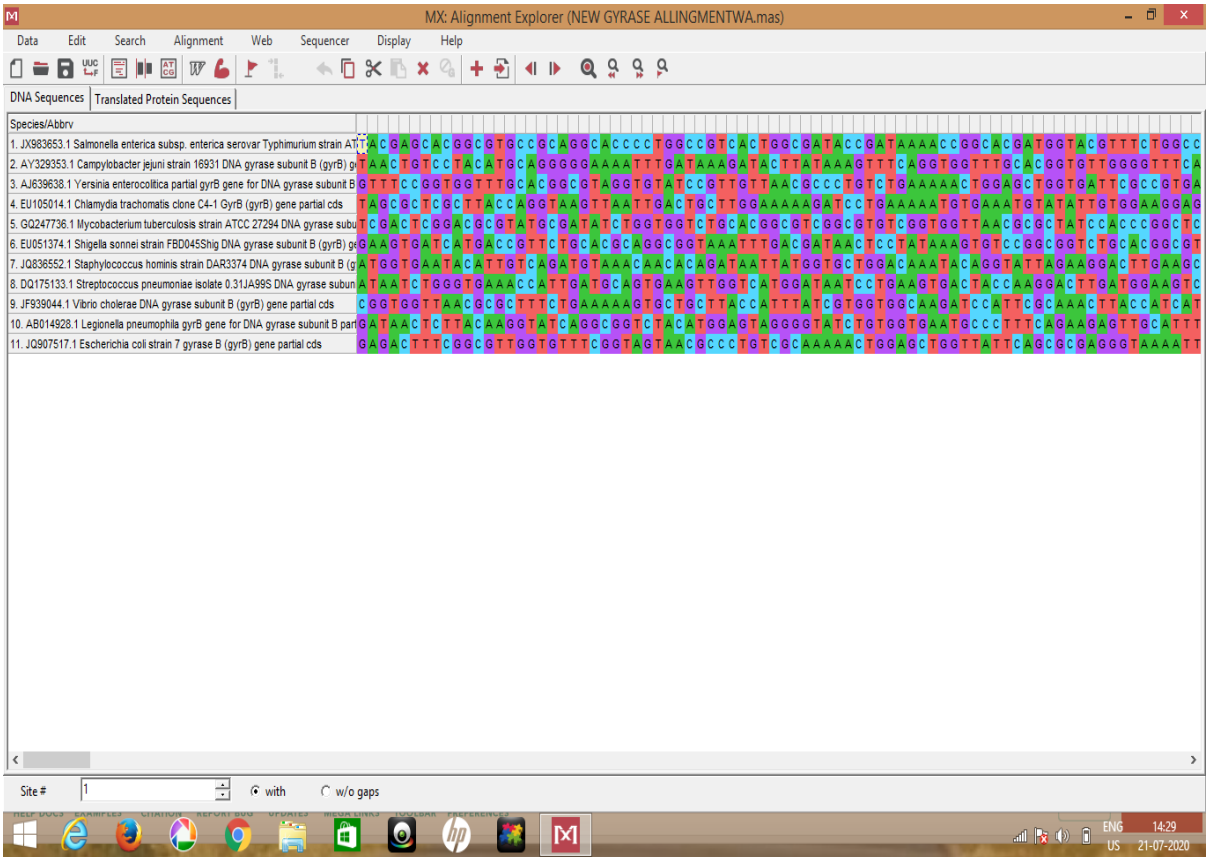

Fig 4.2- The sequence alignment of DNA gyrase gene sequences of pathogenic bacteria by CLUSTALW.

The phylogenetic tree construction of 16S rRNA gene sequences : The phylogenetic tree was constructed by neighbour joining or distance based method based on 16S rRNA of gene sequences describes the probability value 0.20 and evolutionary relatedness among eleven pathogenic bacteria. The result is given below (fig 4.3) with the description of different pathogenic bacteria.

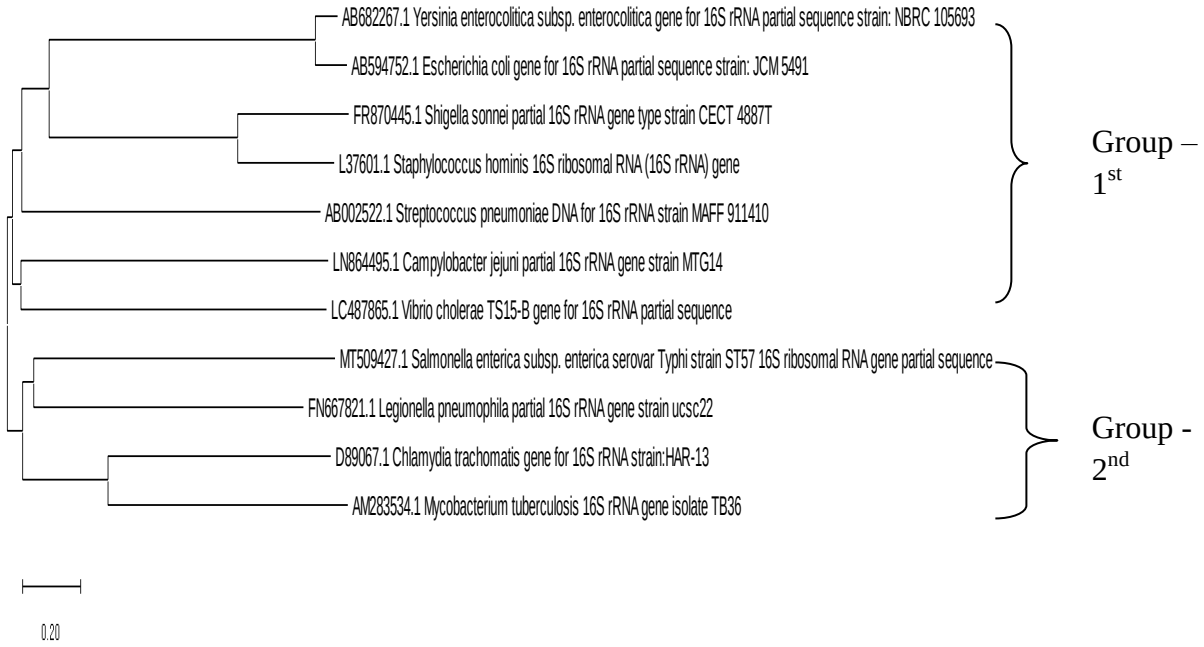

Fig 4.3 – The phylogeny of pathogenic bacteria based on 16S rRNA gene sequences.

Table 4.1 – Pathogenic bacteria of group 1st of 16S rRNA gene with their respective accession numbers, base pairs and associate diseases.

S. No.	Pathogenic bacteria of group -1st	Associate disease	Base pair	NCBI Accession no.
1.	*Yersinia. enterocolitica NRBC 105693*	Yersiniosis	1,468 bp	AB682267.1
2.	*E. coli JCM 5491*	Urinary tract infection	1,464 bp	AB394752.1
3.	*Shigella. sonnei CECT 4887T*	Shigellosis	1,530 bp	FR870445.1
4.	*Staphylococcus hominis*	Skin infection	1,468 bp	L37601.1
5.	*Streptococcus pneumoniae MAF911410*	Pneumonia	1,416 bp	AB002522.1
6.	*Campylobacter. Jejuni MTG14*	Foodborne illness	1,145 bp	LN864495.1
7.	*V. cholerae TS15-B*	Cholera	1,509 bp	LC487865.1

Table 4.2 – Pathogenic bacteria of group 2nd of 16S rRNA gene with their respective accession numbers, base pairs and associate diseases.

S. NO.	Pathogenic bacteria of group – 2nd	Associate disease	Base pair	NCBI Accession no.
1.	*S. enterica subsp. Serovar typhi ST57*	Typhoid fever	1,426 bp	MT509427.1
2.	*Legionella. pneumophila UCSC22*	Legionnaires	1,436 bp	FN667821.1
3.	*C .trachomatis HAR13*	Conjunctivitis	1,548 bp	D89067.1
4.	*M. tuberculosis TB36*	Tuberculosis	1,549 bp	AM283534.1

The phylogenetic tree construction of DNA gyrase gene sequences : The phylogenetic tree was again constructed by neighbour joining or distance based method based on DNA gyrase gene sequences describes the probability value 1 and evolutionary relatedness among eleven pathogenic bacteria. The result given below (fig4.4) with the description of different pathogenic bacteria.

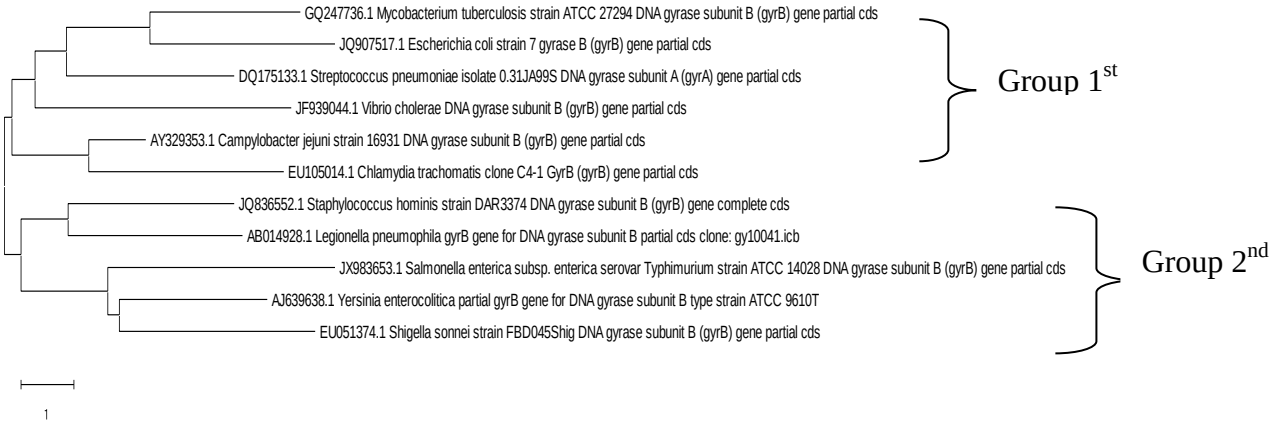

Fig 4.4- The phylogeny of pathogenic bacteria based on DNA gyrase gene sequences.

Table 4.3 –Pathogenic bacteria of group 1st of DNA gyrase gene with their respective accession numbers, base pairs, and associate diseases.

S. No.	Pathogenic bacteria of group – 1st	Associate disease	Base pair	NCBI Accession no.

1.	*Mycobacterium tuberculosis ATCC 27294*	Tuberculosis	1,256 bp	GQ247736.1
2.	*E. coli 7*	Urinary tract infection	1,119 bp	JQ907517.1
3.	*Streptococcus. pneumoniae 0.31JA99S*	Pneumonia	1,992 bp	DQ175133.1
4.	*V. cholerae*	Cholera	1,083 bp	JF939044.1
5.	*Campylobacter jejuni 16931*	Foodborne illness	1,221 bp	AY32935.1
6.	*Chlamydia trachomatis C4-1*	Conjunctivitis disease in cornea	1,180 bp	EU105014.1

Table 4.4 –Pathogenic bacteria of group 2nd of DNA gyrase gene with their respective accession numbers, base pairs and associate diseases.

S. No.	**Pathogenic bacteria of group -2nd**	**Associate disease**	**Base pair**	**NCBI Accession no.**
1.	*Staphylococcus hominis DAR3374*	Skin infection	1,935 bp	JQ836552.1
2.	*Legionella pneumophila gy10041.icb*	Legionnaires	1,167 bp	AB14928.7
3.	*S. enterica subsp. Serovar typhimurium ATCC14028*	Typhoid fever	1,037 bp	JX983653.1
4.	*Yersinia enterocolitica ATCC 96010T*	Yersiniosis	1,038 bp	AJ639638.1
5.	*Shigella sonnei FBD045shig*	Shigellosis	1,256 bp	EU051374.1

Phylogenetic trees are commonly used for optical representation in the life sciences and the most essential optical representation in evolutionary studies. After the victory of phylogenetic tree construction, results clarified that position of bacteria are different in both the phylogenetic trees fig 4.3 and fig 4.4. Closely related species represents that they are sharing

common ancestors and sharing same genes among their peer groups. The representation of fig 4.3 clarify that *Yersinia enterocolitica* strain shares the most recent common ancestor with *E.coli* strain, *Shigella sonnei* strain and *Staphylococcus hominis* strain are also sharing most recent common ancestor. The tree representation of fig 4.4 explains that *Yersinia enterocolitica* strain shares the closely relationship with *Shigella sonnei* strain and same as *Campylobater jejuni* strain is closely related to *Chlamydia trachomatis* strain. Grouping in phylogeny represents the last common ancestor, origin of root. As shown in table 4.1 the group 1st of pathogenic bacteria which are the *Yersinia enterocolitica* strain, *Escherichia coli* strain, *Shigella sonnei* strain, *Staphylococcus hominis* strain, *Streptococcus pneumoniae* strain, *Campylobacter jejuni* strain, *Vibrio cholerae* strain are sharing same root, somewhat sharing common ancestor with each other in phylogenetic tree of 16S rRNA genes and the phylogenetic tree of DNA gyrase gene represents the group 1st group of pathogenic bacteria in table 4.3 that *Mycobacterium tuberculosis* strain, *Escherichia coli* strain, *Streptococcus pneumoniae* strain, *Vibrio cholerae*, *Campylobacter jejuni* strain, *Chlamydia trachomatis* strain are sharing the distantly related common ancestor with one another. After the comparison of phylogenetic representation of both 16S rRNA and DNA gyrase gene, result interprets that estimation of evolutionary history and relationships cannot be done only on 16S rRNA. Both the tree shows the changes in position of disease causing bacteria (Naushad et al. 2016).

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Conclusion and future prospects

Comparison of phylogenies on the basis of 16S rRNA and DNA gyrase genes sequences provided the details about the evolutionary relationship of disease causing bacteria. Construction of phylogenetic tree is one of the chief techniques of molecular characterization to understand about the evolutionary relationship among different organism. According to the scientists, the phylogenies can explain the disease at genetic level and provides the details about the history, current situation and afterwards of pathogens. According to many researchers phylogenetic methods are reliable for identification of pathogens. The accomplishment has shown that the all eleven pathogenic bacteria have different position in phylogenetic tree of 16S rRNA and DNA gyrase. The pathogenic bacterial strains have acquired the positions according to their gene sharing with the fellow groups and their common ancestors. The future of phylogeny will be the considerable data to preserve its relations with ancient root and prompt biological data through species names and evolution. Pathogens have different distribution; different mode of action, different rate of growth can be understood by phylogeny. This justify different characters at gene and geographic level by the comparison of location of isolated sample to investigate source of spread and molecular methods can be applied to check the evolution, events, spreading of new pathogens. Many researchers thought that comparative methods should do better work for collecting multiple data into a systematic framework. Projects with common aims, like evaluating when and why an ancestry undergoes speciation, are better united than separate. Comparative set of data help us to measure about an evolving genus from one generation to the next. Some comparative methods also assimilate information from earth science documentation especially fossils, but also other moderate and periodic events in the globe’s history. In anthropology recent techniques developed for phylogenetics have been tried successfully to languages in the exact identical ways as creating species phylogenetic trees grounded on genomes. PCMs are progressively used in fields apart from evolutionary biology such as community ecology, anthropology, linguistics, paleobiology. These methods have becoming powerful recently due to the support of data resources and computational power to construct larger and better phylogenies.

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